Figure 1: Localisation of mutations in Fermichamp HXT3

Glu 414   Gln
Gly 415   Asn

Leu471 Ile

Ile449 Val

Ile392 Val
Tyr389 Trp
Leu388 Met

Met324 Ile

Thr200 ala

Ile209 Val

★   Mutated amino acid
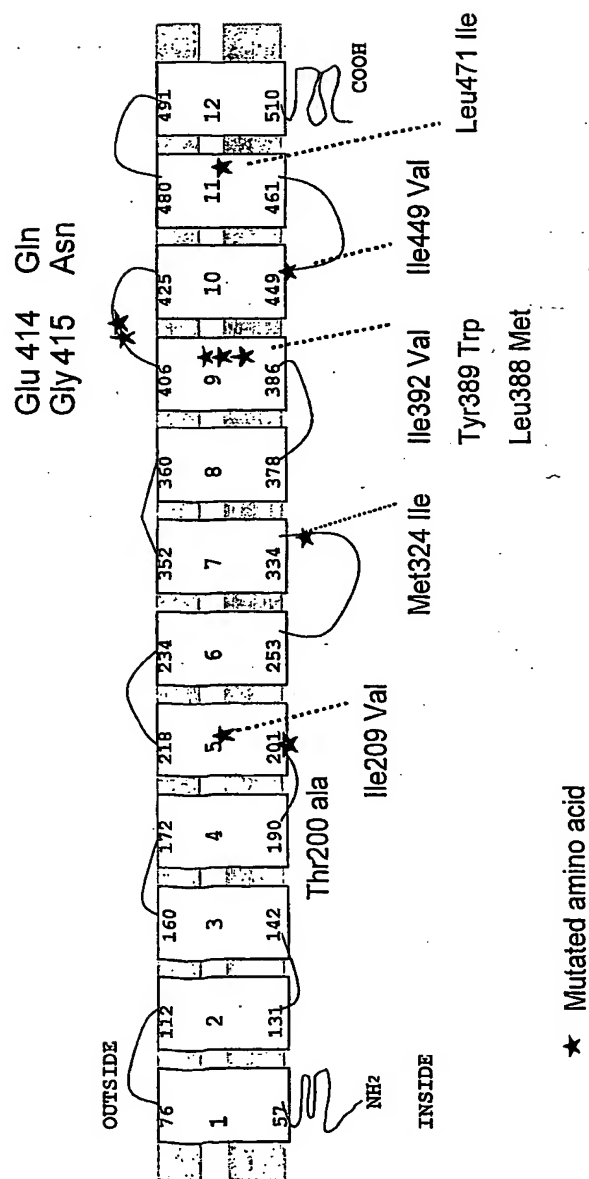
Figure 2A Construction of V5 strains with integrated HXT3 genes

HXT3 integration in V5 hxt1-7Δ strain

HXT3-6-7 locus in wild type V5 strain

Chromosomal integration by homologous recombination in V5 hxt1-7Δ yeast strain

Fermichamp or V5 HXT3 PCR amplification

Integrated HXT3 gene
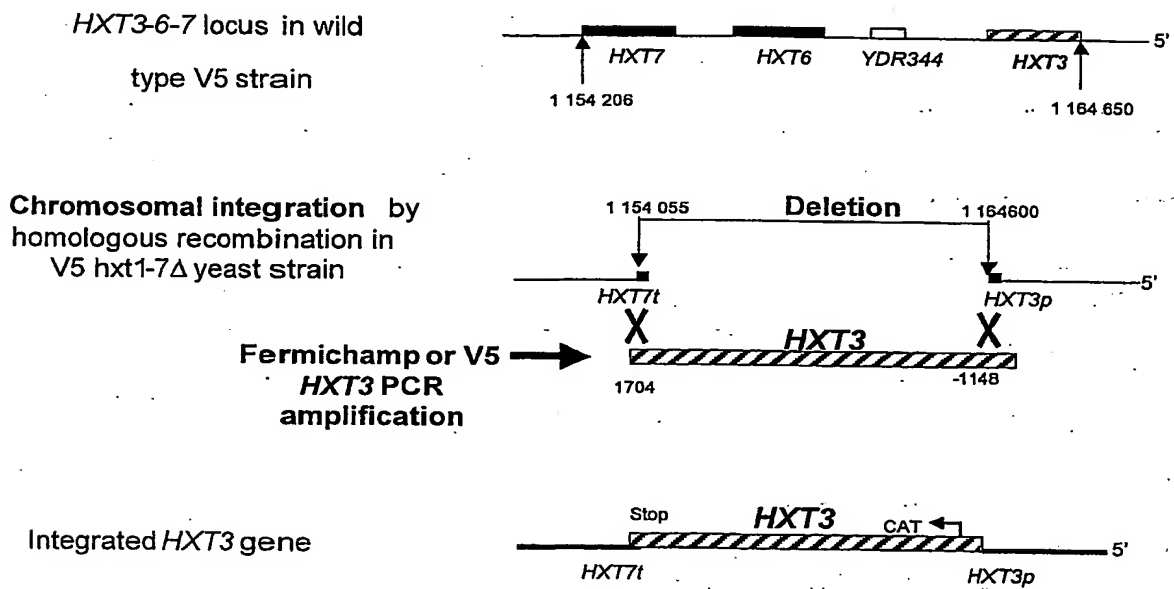
Figure 2B : *HXT3* ORF cloning in multicopy plasmid p4H7

HXT3 *from* **Fermichamp or V5 PCR amplification**

ATG   Stop

HXT3

Cloning in p4H7 plasmid by homologous recombination in yeast

BamHI EcoRI

HXT7pro   CYC1term

p4H7

BamHI + EcoRI

Mix and transformation of yeast
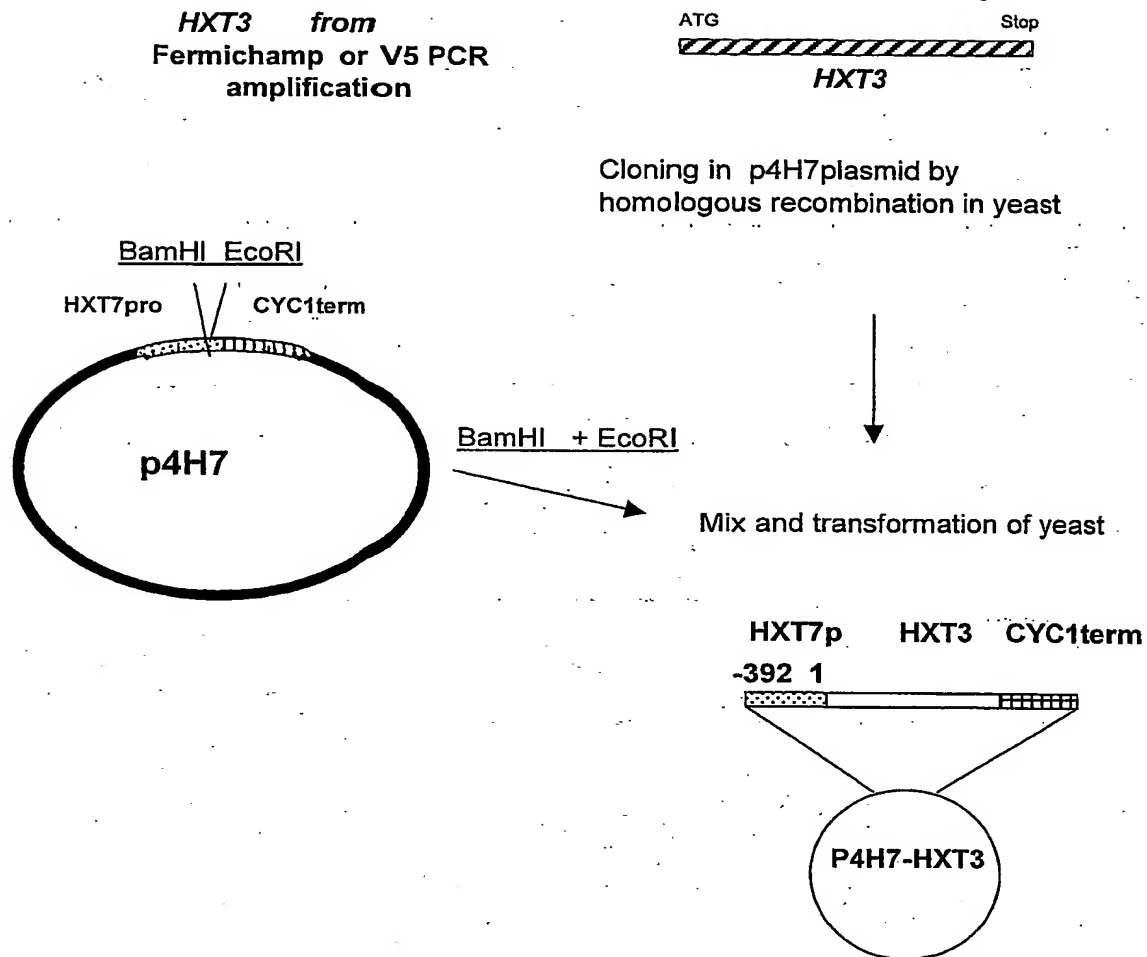
HXT7p   HXT3   CYC1term

-392 1

P4H7-HXT3

Figure 3A glucose and fructose utilisation by *HXT3* (**V5** or **Fmp**) single copy
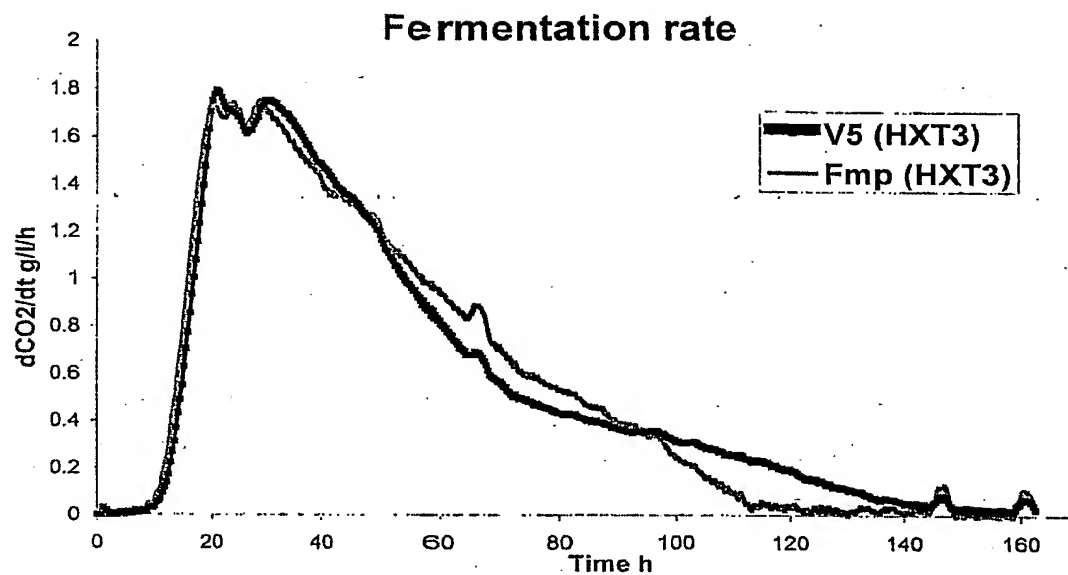gene expression

Glucose & Fructose uptake

Legend:
- V5 (HXT3) Glc
- V5 (HXT3) Frc
- Fmp (HXT3) Glc
- Fmp (HXT3) Frc

Y-axis: Sugar g/l
X-axis: CO_2 g/l
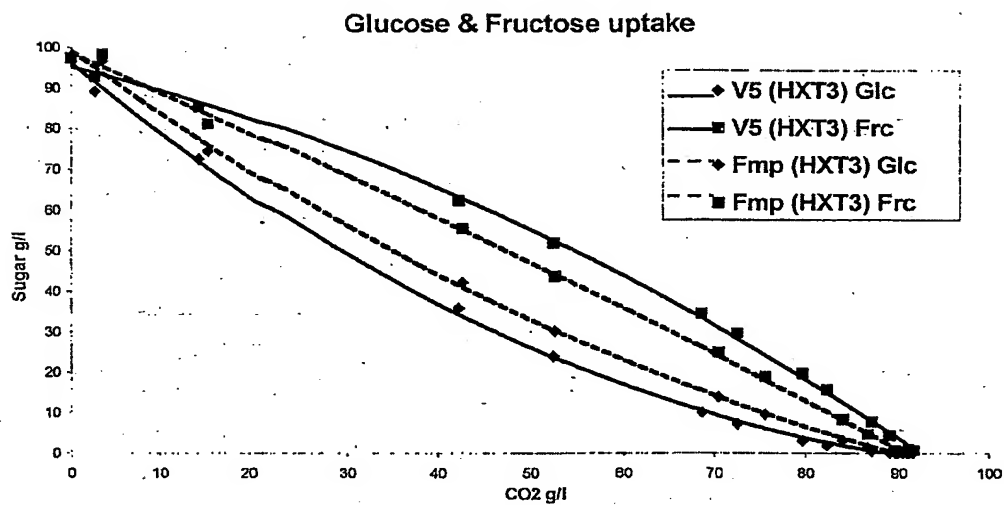
Figure 3B : glucose/fructose ratio of *HXT3* (**V5** or **Fmp**) single copy gene expression

Glucose/Fructose ratio
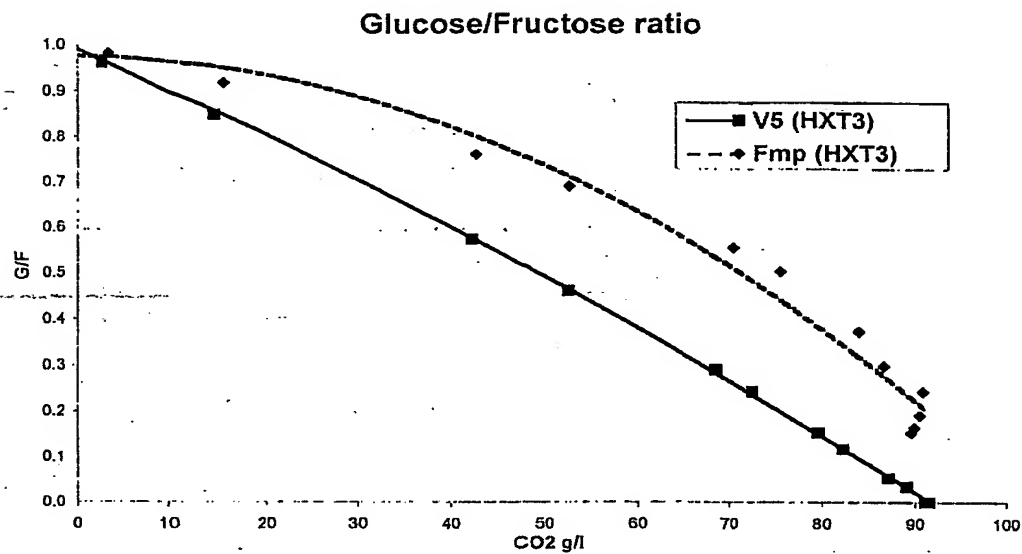
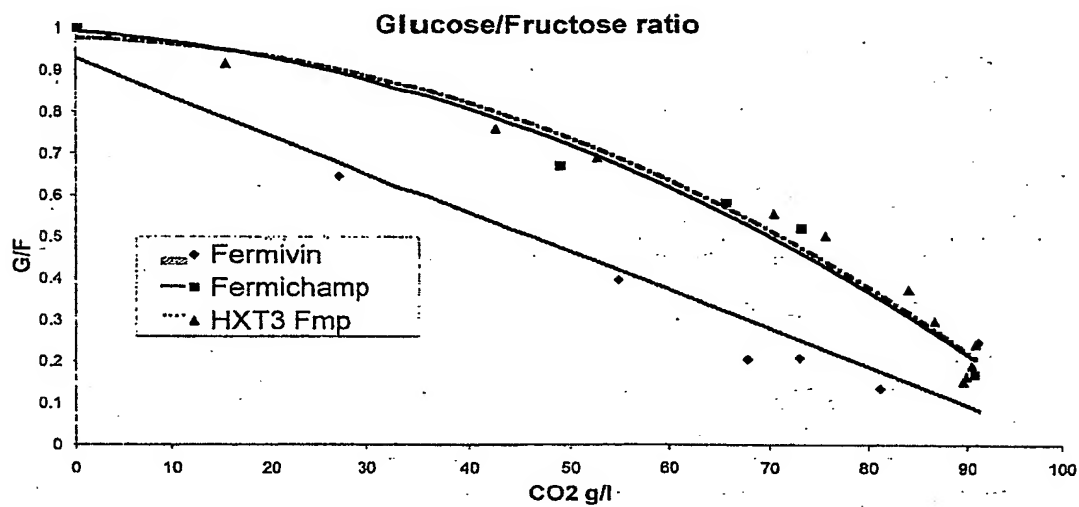Figure 3C : fermentation rate of *HXT3* (**V5** or **Fmp**) single copy gene expression

Figure 3D

Comparison of Glucose / Fructose ratio between Fermichamp & *HXT3* (**V5** or
Fmp) single copy gene expression

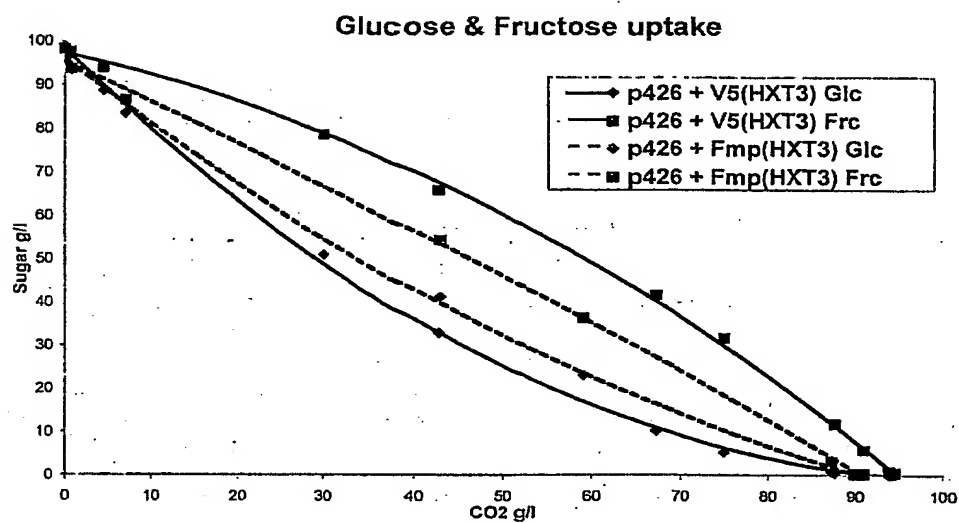Glucose/Fructose ratio
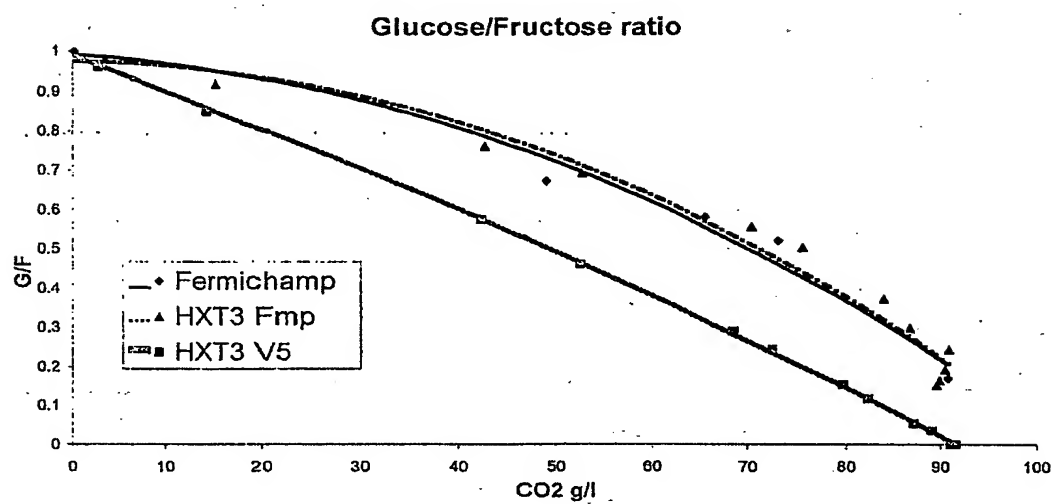
Figure 3 E : Comparison of Glucose / Fructose ratio between Fermichamp, Fermivin & *HXT3* **Fmp** single copy gene expression

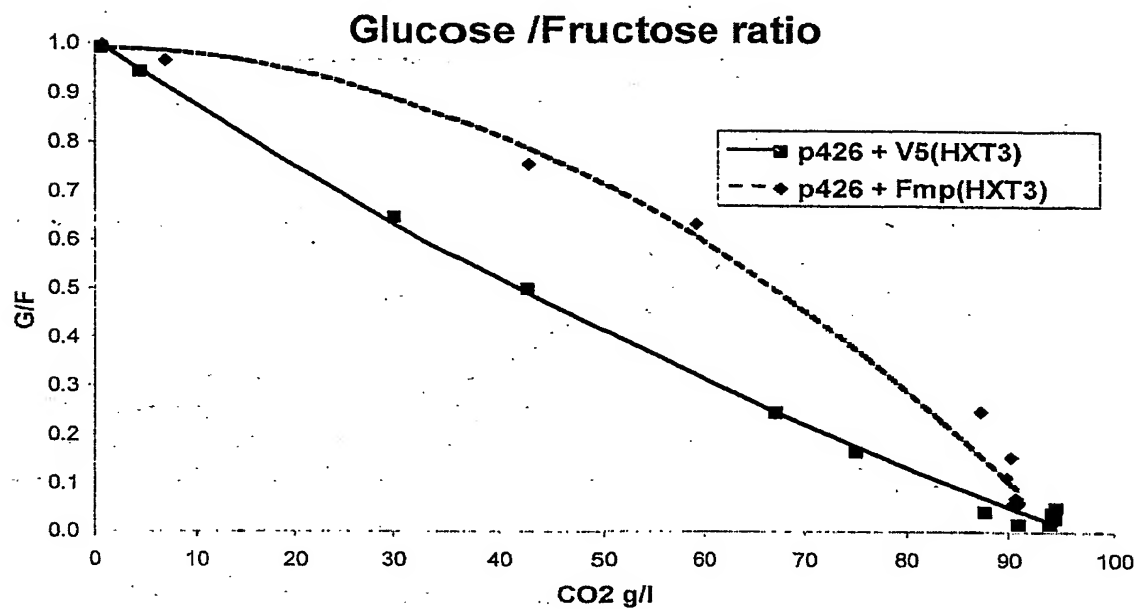Glucose/Fructose ratio

Figure 4A : glucose and fructose utilisation by multicopy overexpression of *HXT3*
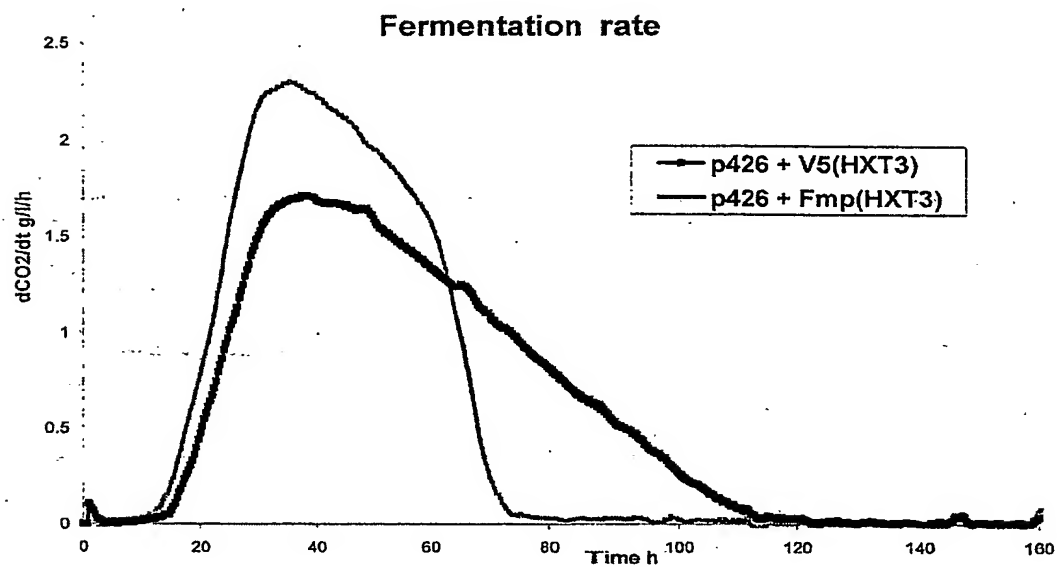(V5 or Fmp)

Glucose & Fructose uptake

Figure 4B : glucose/fructose ratio by multicopy overexpression of *HXT3* (V5 or Fmp)

Glucose /Fructose ratio

Figure 5 : Multicopy overexpression of *HXT3* (V5 or Fmp) on Glucose + Fructose
(50/50) must (200g/l)

Fermentation rate

Figure 6A : Multicopy overexpression of *HXT3* (V5 or Fmp) on pure Fructose must
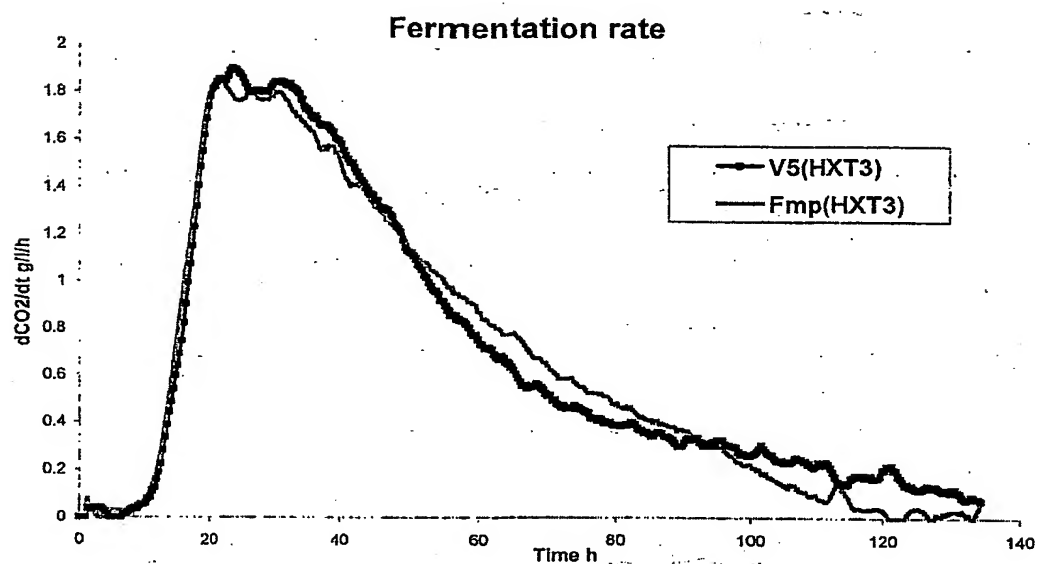(200g/l)

Fermentation rate
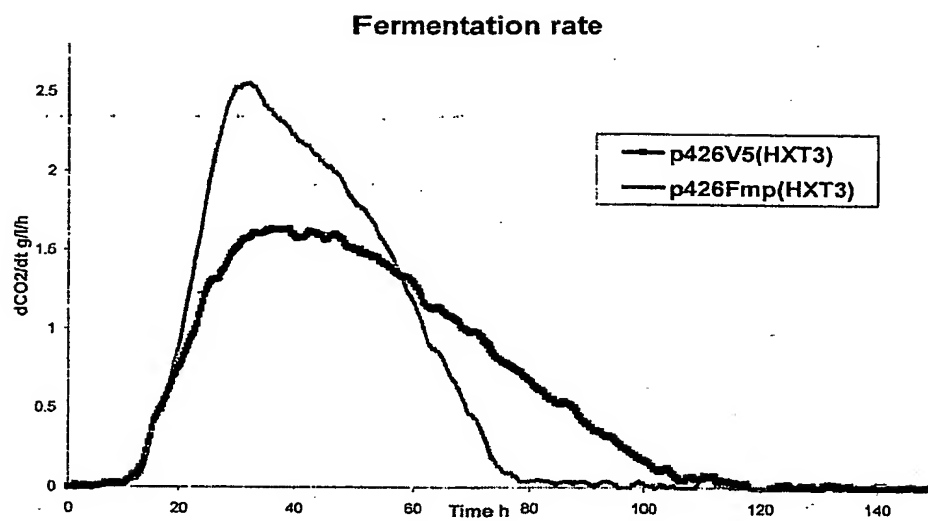
Figure 6B : Multicopy overexpression of *HXT3* (V5 or Fmp) on pure Glucose must
(200g/l)

Fermentation rate
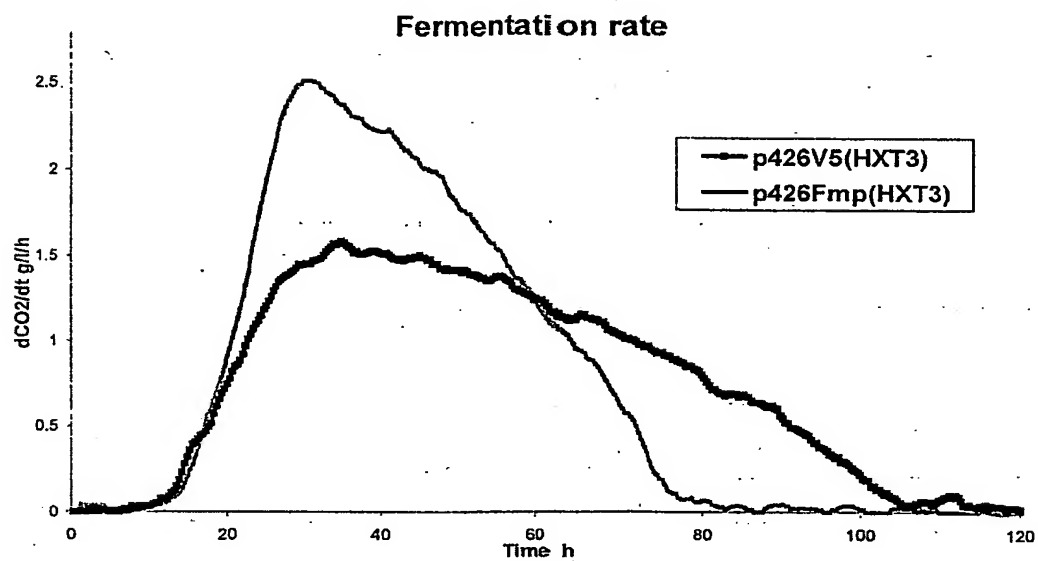
Figure 7A : Single copy expression of *HXT3* (V5 or Fmp) on pure Fructose must (200g/l)

Fermentation rate

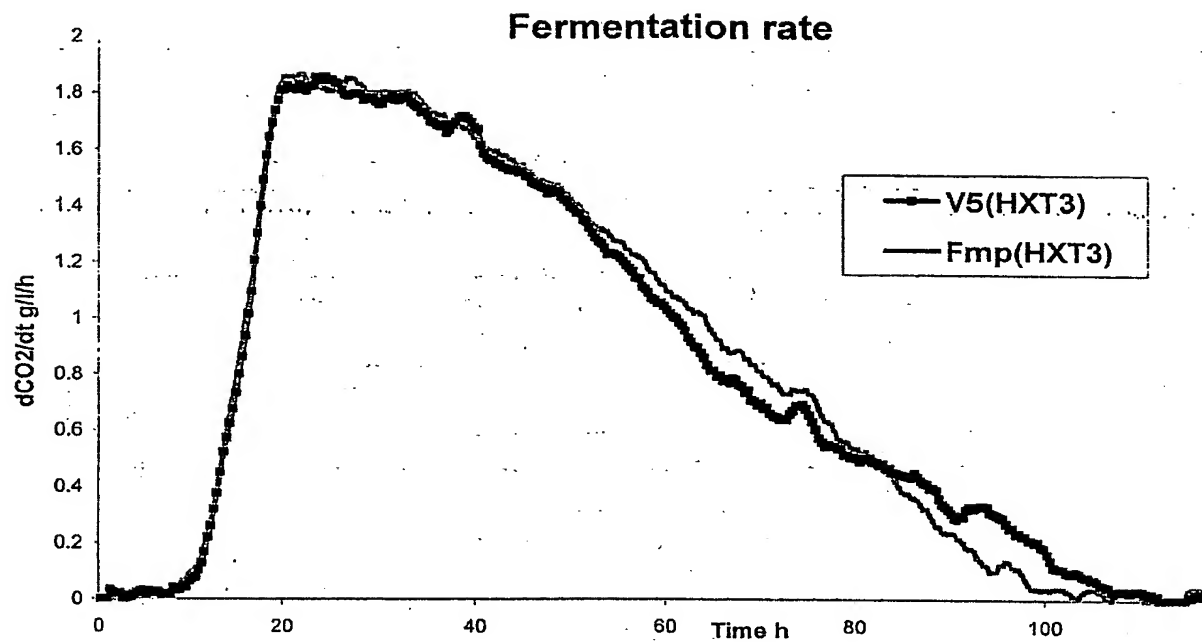Figure 7B : Single copy expression of *HXT3* (V5 or Fmp) on pure Glucose must (200g/l)

Figure 8: Construction of strains that contain a single, inactive, HXT3 gene, general scheme of strains construction
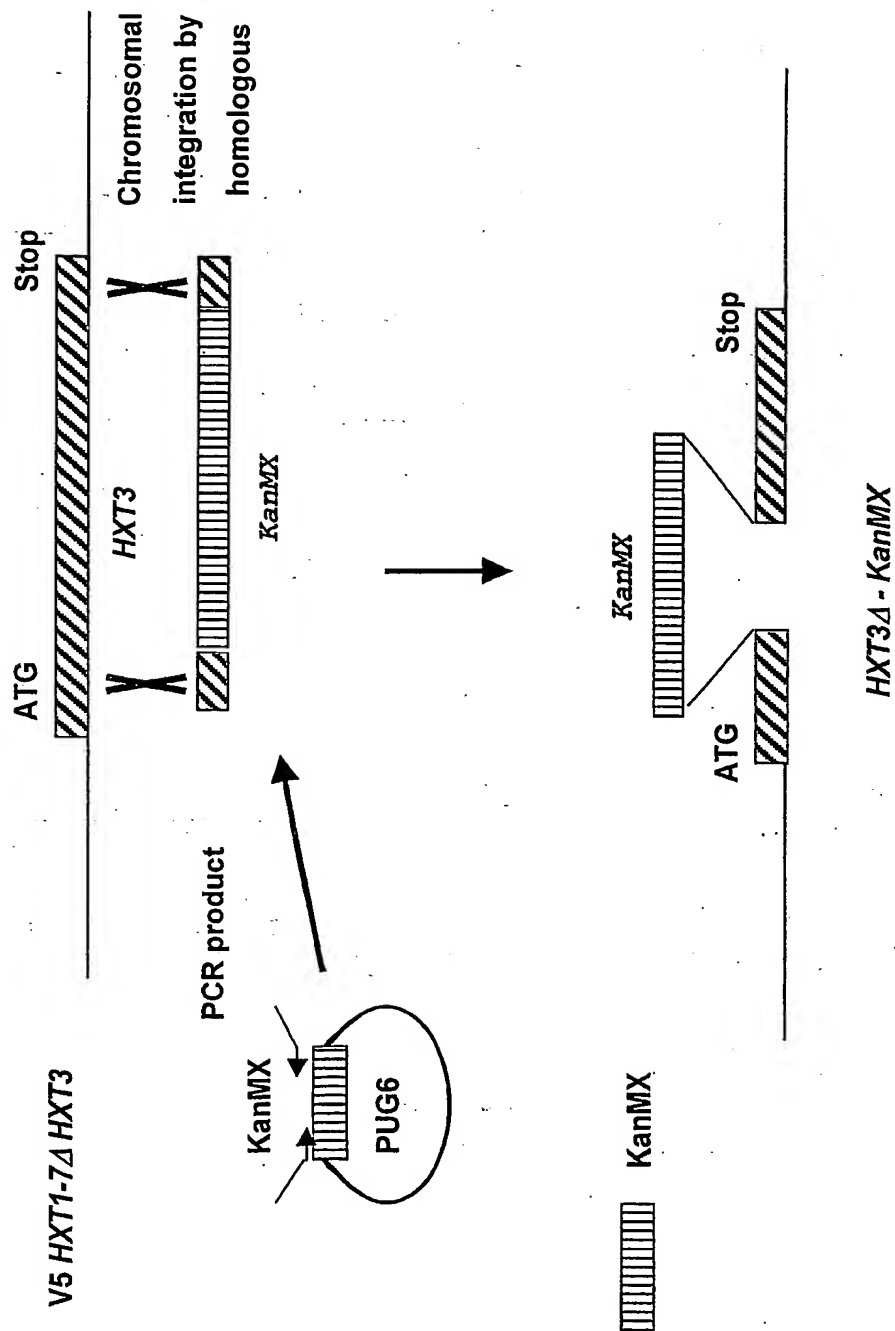
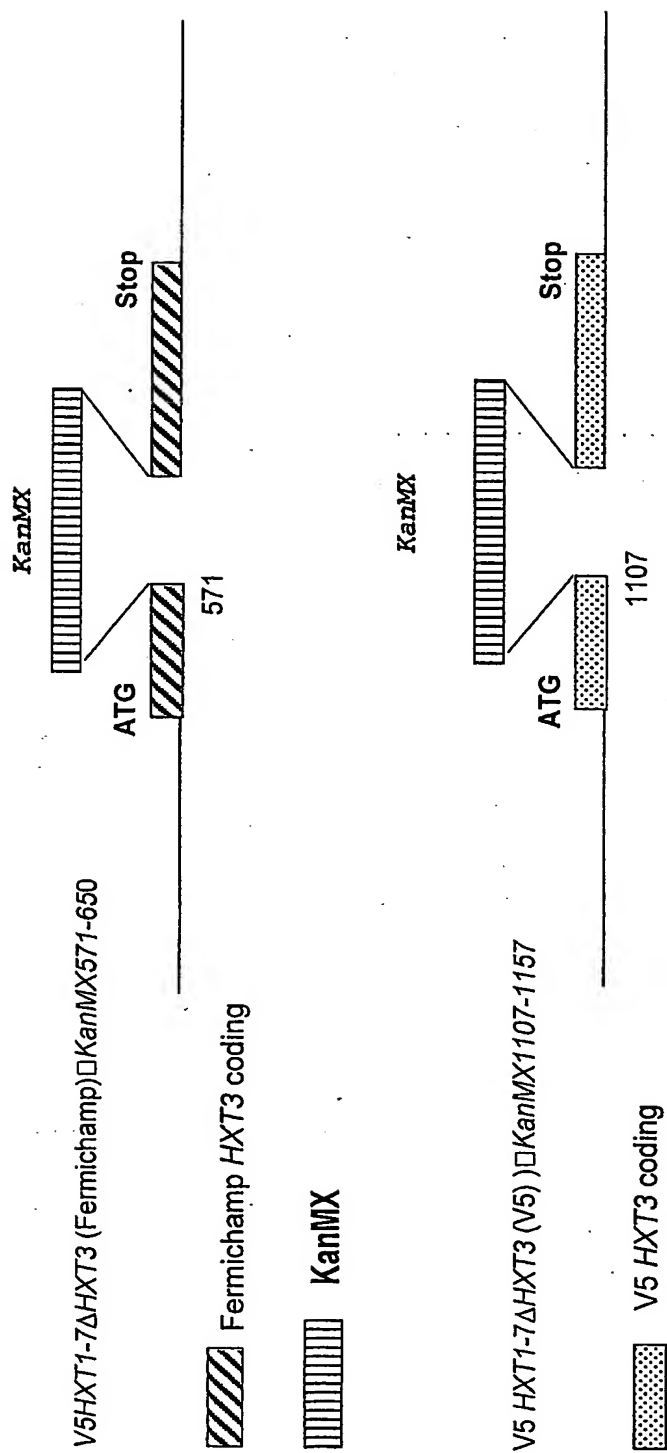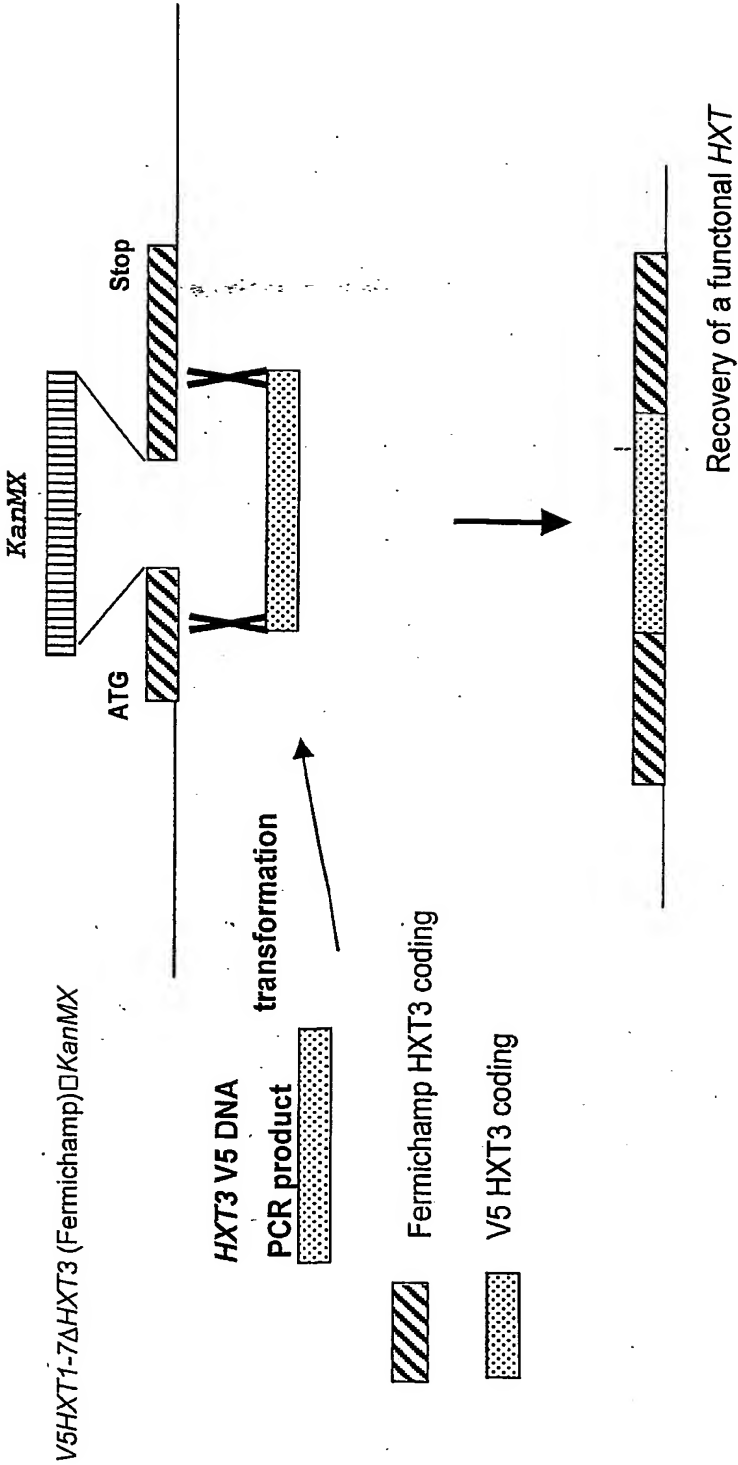Figure 9:Constructed strains comprising a single, inactive HXT3 gene

Figure 10: Construction of strains expressing *HXT3* chimera: principle of construction

Figure 11: Chimeric *HXT3* proteins expressed
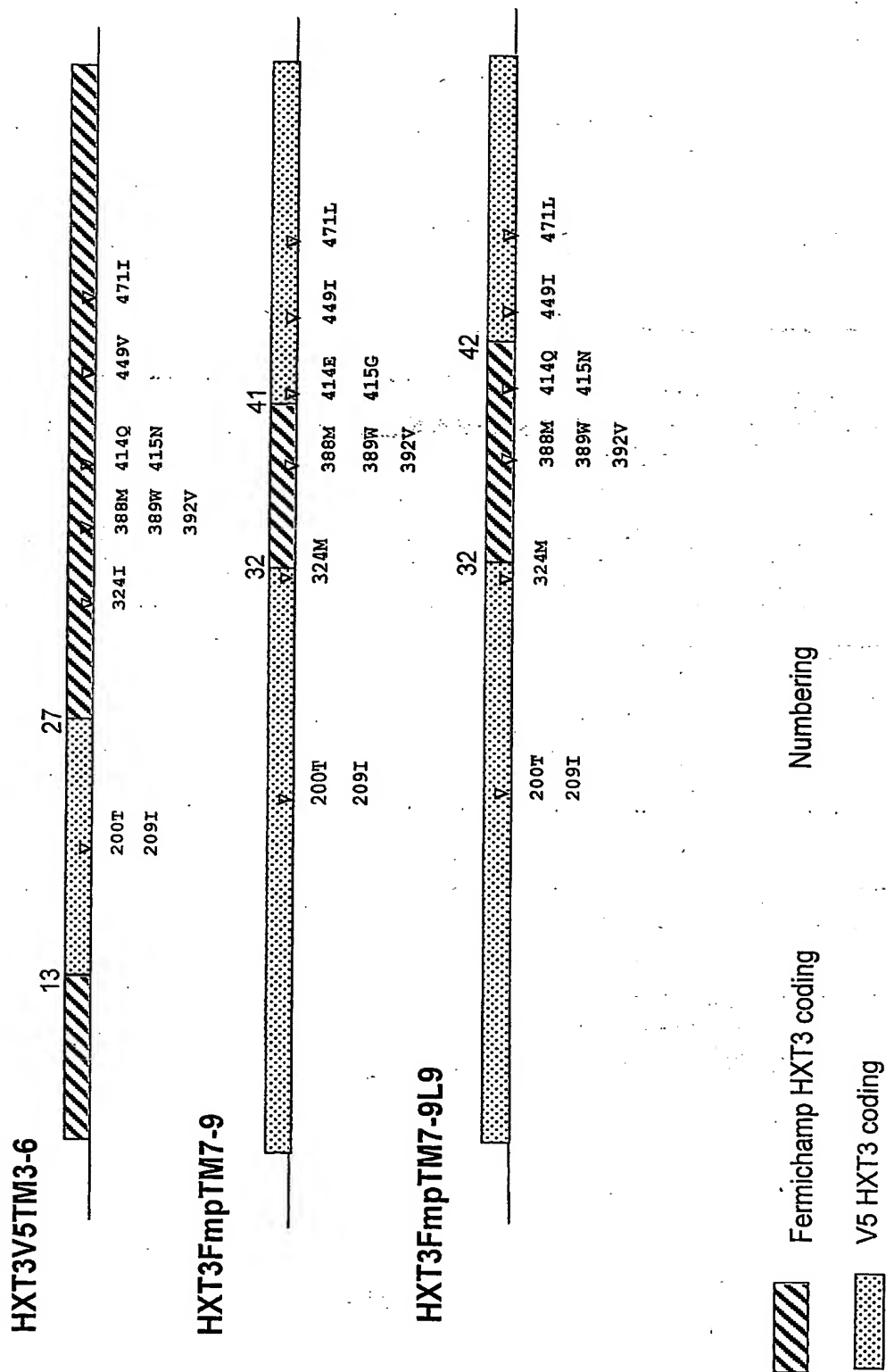
Figure 12: Mutated *HXT3* Fermichamp proteins (point mutations)

HXT3FmpT200

200T

HXT3FmpI209
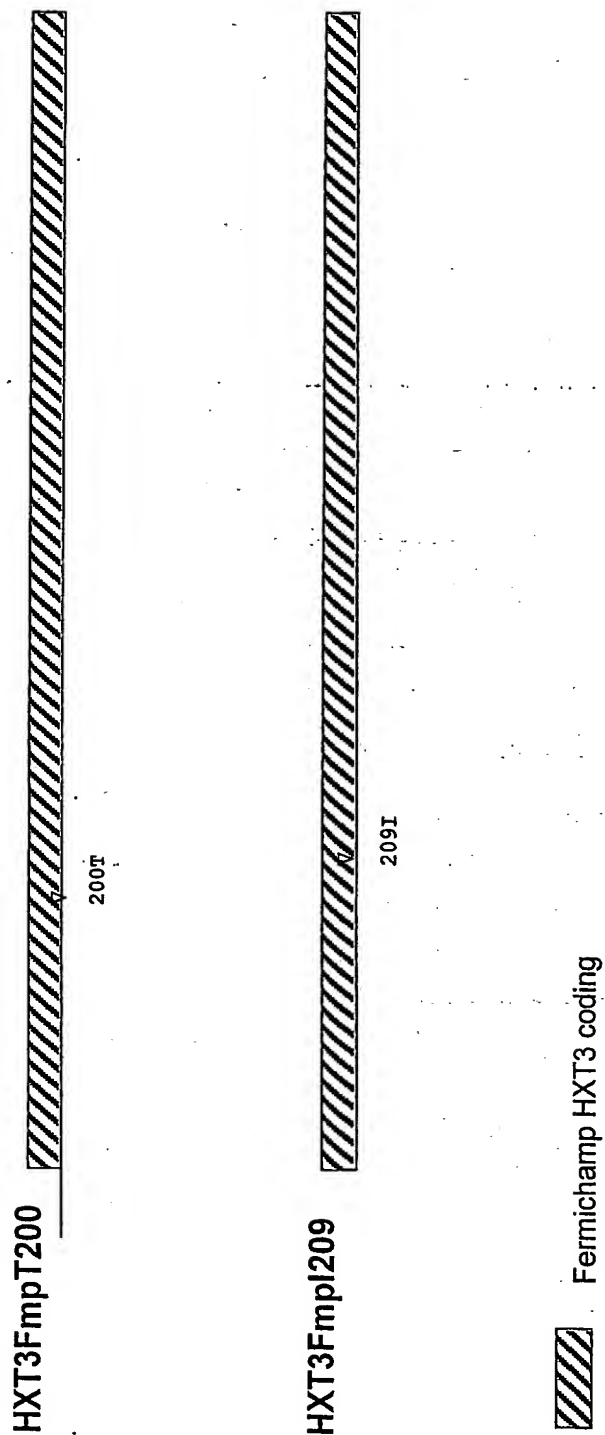
209I

Fermichamp HXT3 coding

Figure 13: Glucose-Fructose ratio evolution during alcoholic fermentation
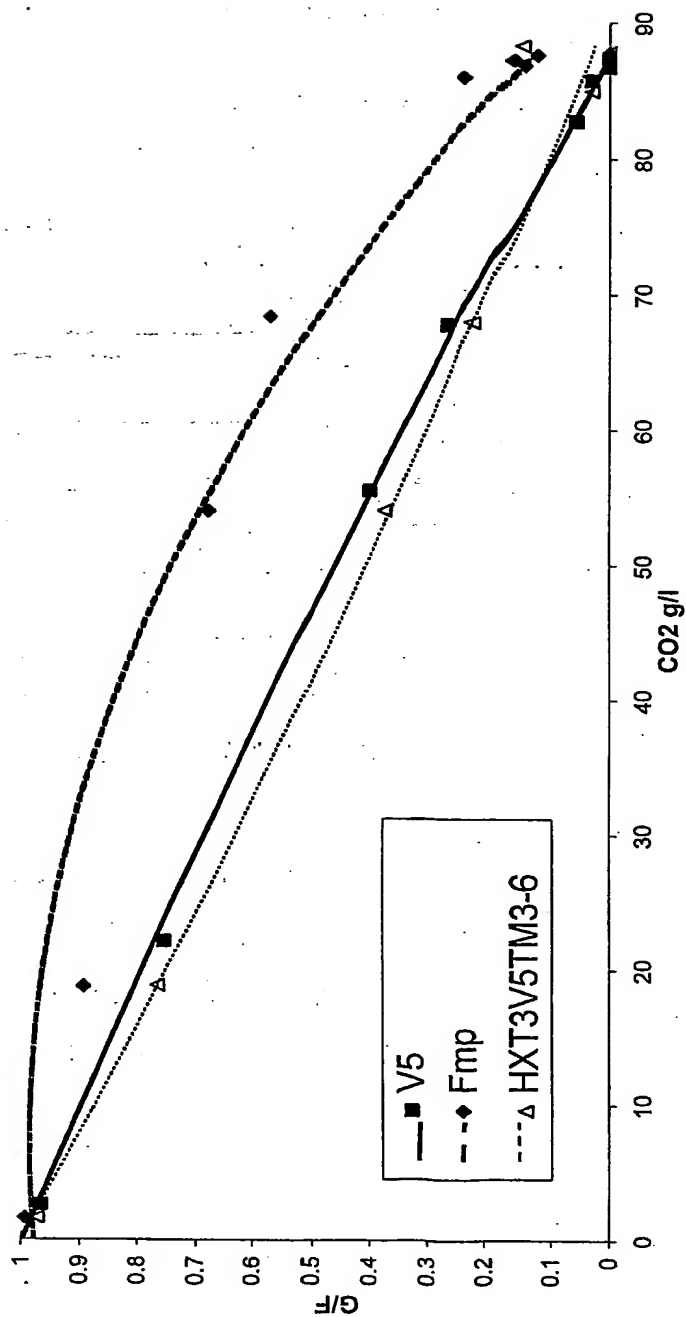
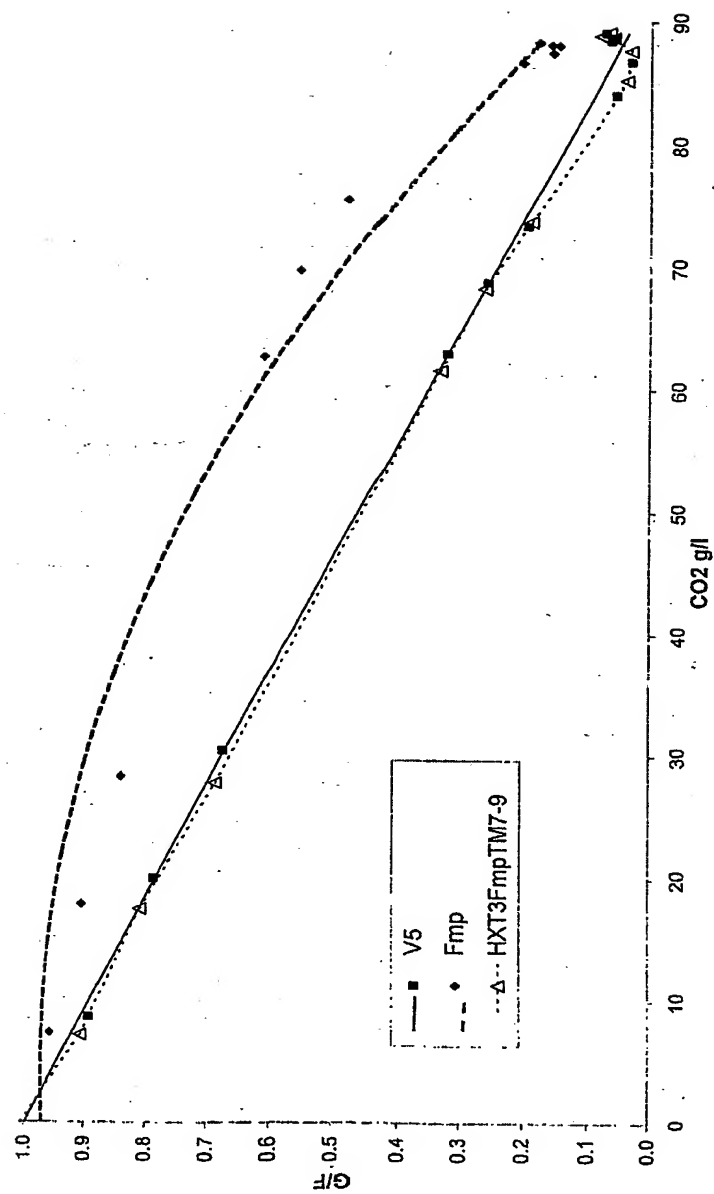Figure 14: Glucose / Fructose ratio evolution during alcoholic fermentation

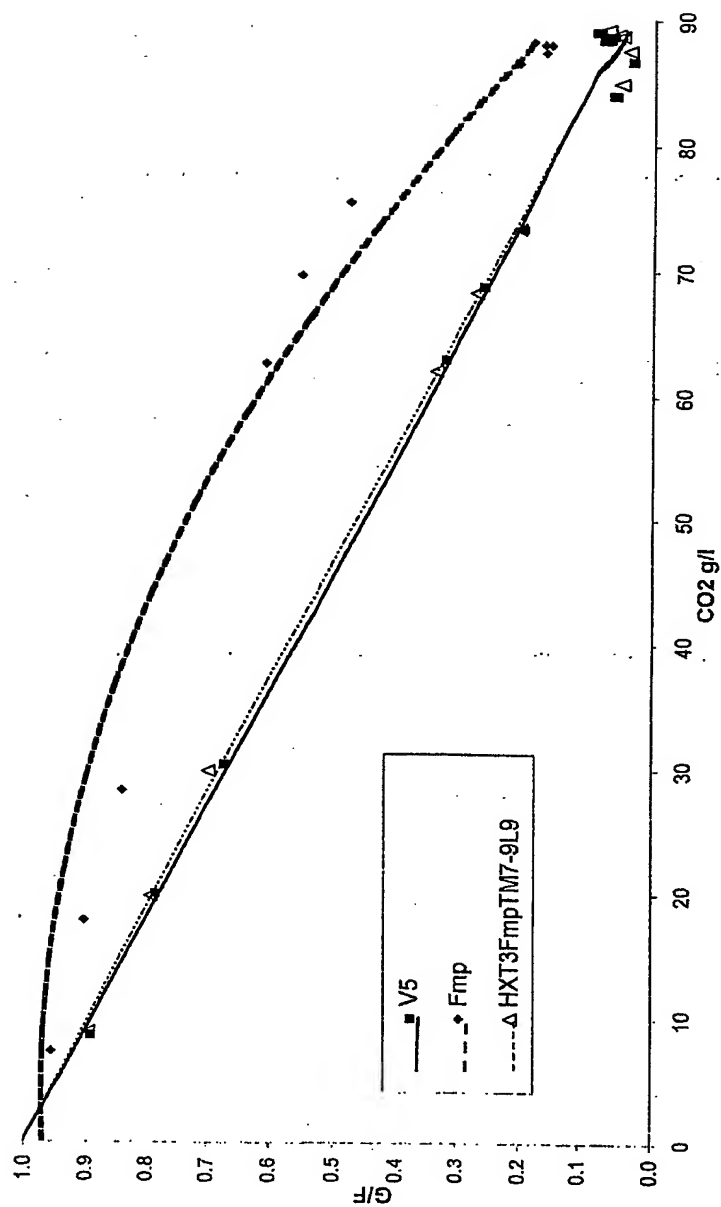Figure 15: Glucose/Fructose ratio evolution during alcoholic fermentation

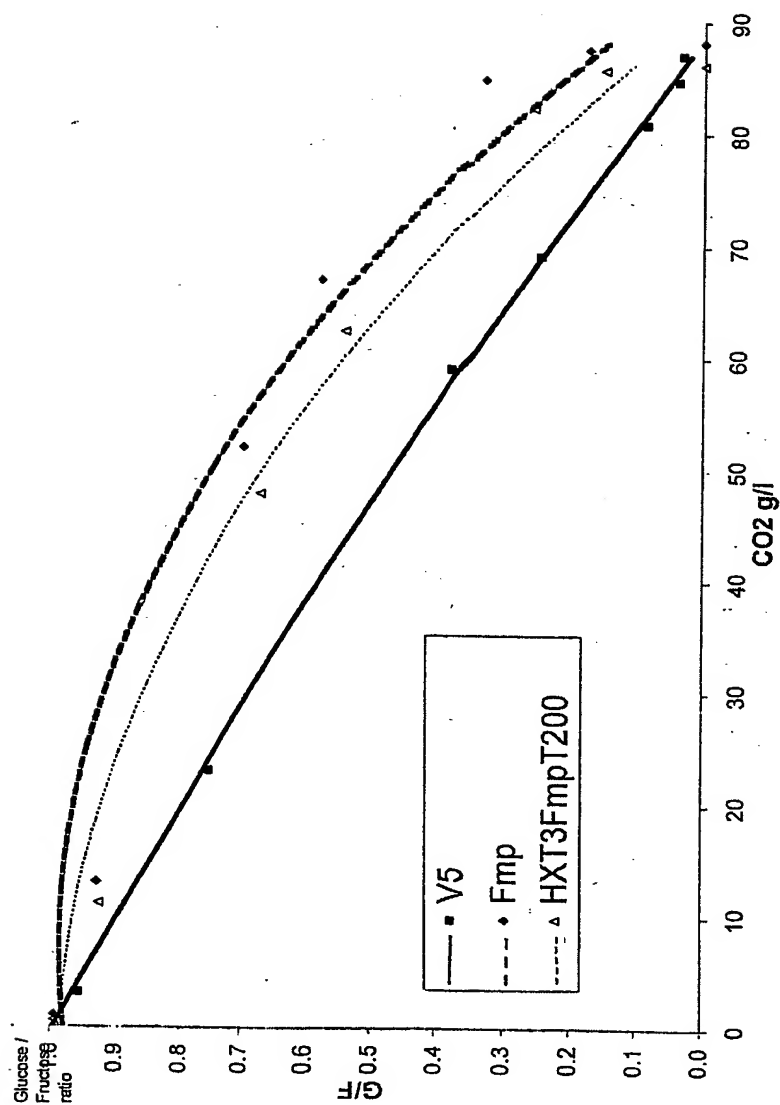Figure 16: Glucose/Fructose ratio evolution during alcoholic fermentation

Figure 17: Glucose/Fructose ratio evolution during alcoholic fermentation